

Methods in Cell Biology

Edited by

DAVID M. PRESCOTT

VOLUME XV

Methods in Cell Biology

Edited by

DAVID M. PRESCOTT

DEPARTMENT OF MOLECULAR, CELLULAR AND
DEVELOPMENTAL BIOLOGY
UNIVERSITY OF COLORADO
BOULDER, COLORADO

VOLUME XV

ACADEMIC PRESS • New York San Francisco London
An imprint subsidiary of Harcourt Brace Jovanovich, Publishers

**COPYRIGHT © 1977, BY ACADEMIC PRESS, INC.
ALL RIGHTS RESERVED.**

**NO PART OF THIS PUBLICATION MAY BE REPRODUCED OR
TRANSMITTED IN ANY FORM OR BY ANY MEANS, ELECTRONIC
OR MECHANICAL, INCLUDING PHOTOCOPY, RECORDING, OR ANY
INFORMATION STORAGE AND RETRIEVAL SYSTEM, WITHOUT
PERMISSION IN WRITING FROM THE PUBLISHER.**

ACADEMIC PRESS, INC.
111 Fifth Avenue, New York, New York 10003

United Kingdom Edition published by
ACADEMIC PRESS, INC. (LONDON) LTD.
24/28 Oval Road, London NW1

LIBRARY OF CONGRESS CATALOG CARD NUMBER: 64-14220

ISBN 0-12-564115-X

PRINTED IN THE UNITED STATES OF AMERICA

LIST OF CONTRIBUTORS

Numbers in parentheses indicate the pages on which the authors' contributions begin.

- BARBRO ANDERSSON, Department of Pathology, School of Medicine, Leahi Hospital, Honolulu, Hawaii (435)
- STRATIS AVRAMEAS, Immunocytochemistry Unit, Pasteur Institute, Paris, France (387)
- KARYN E. BIRD, Webb-Waring Lung Institute, University of Colorado Medical Center, Denver, Colorado (1)
- L. M. BLACK, Department of Genetics and Development, University of Illinois, Urbana, Illinois (407)
- MICHEL BORNENS, Department of Molecular Biology, Pasteur Institute, Paris, France (163)
- JOSEPH E. CUMMINS, Department of Plant Sciences, The University of Western Ontario, London, Ontario, Canada (445)
- RICHARD L. DAVIDSON, Division of Human Genetics, Children's Hospital Medical Center, and Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts (325)
- ALAN W. DAY, Department of Plant Sciences, The University of Western Ontario, London, Ontario, Canada (445)
- T. EGE, Institute for Medical Cell Research and Genetics, Medical Nobel Institute, Karolinska Institute, Stockholm, Sweden (339)
- A. J. FABER, Swiss Institute for Experimental Cancer Research, Lausanne, Switzerland (127)
- S. FAKAN, Swiss Institute for Experimental Cancer Research, Lausanne, Switzerland (127)
- PARK S. GERALD, Division of Human Genetics, Children's Hospital Medical Center, and Department of Pediatrics, Harvard Medical School, Boston, Massachusetts (325)
- PETER J. GOLDBLATT, Department of Pathology, University of Connecticut Health Center, Farmington, Connecticut (371)
- NICHOLAS K. GONATAS, Department of Pathology (Division of Neuropathology), University of Pennsylvania, School of Medicine, Philadelphia, Pennsylvania (387)
- H. HAMBERG, Department of Pathology, Sabbatsberg Hospital, Stockholm, Sweden (339)
- R. HANCOCK, Swiss Institute for Experimental Cancer Research, Lausanne, Switzerland (127)
- REINHOLD G. HERRMANN, Institut für Botanik der Universität Düsseldorf, Düsseldorf, West Germany (177)
- RICHARD H. HILDERMAN, Department of Biochemistry, Clemson University, Clemson, South Carolina (371)
- MASAKATSU HORIKAWA, Division of Radiation Biology, Faculty of Pharmaceutical Sciences, Kanazawa University, Kanazawa, Japan (97)
- I. R. JOHNSTON, Biochemistry Department, University College, London, England (277)
- JOSEPH R. KATES, Department of Microbiology, State University of New York at Stony Brook, Stony Brook, New York (359)
- R. R. KAY, Imperial Cancer Research Fund, London, England (277)
- CLAUDIA KENT, Department of Biochemistry, Purdue University, West Lafayette, Indiana (289)
- ALOIS KNOSEL, Department of Pharmacology and Biochemistry, School of Veterinary Medicine, University of Zürich, Zürich, Switzerland (89)

- LEROY KUEHL, Department of Biochemistry, University of Utah, Salt Lake City, Utah (79)
- CLIVE C. KUENZLE, Department of Pharmacology and Biochemistry, School of Veterinary Medicine, University of Zürich, Zürich, Switzerland (89)
- M. LANNEAU, I.N.R.A. - Laboratoire de Physiologie de la Reproduction, Nouzilly, France (55)
- TERESA H. LIAO, Department of Chemistry, Nutritional Science Program, University of Maryland, College Park, Maryland (381)
- M. LOIR, I.N.R.A. - Laboratoire de Physiologie de la Reproduction, Nouzilly, France (55)
- JOSEPH J. LUCAS, Department of Microbiology, State University of New York at Stony Brook, Stony Brook, New York (359)
- F. MACHICAO, Max-Planck-Institut für Biochemie, Martinsried, West Germany (149)
- GERARDO MARTÍNEZ-LÓPEZ, Virus Laboratory, Instituto Colombiano Agropecuario, Bogotá, Colombia (407)
- MARVIN L. MEISTRICH, Section of Experimental Radiotherapy, The University of Texas System Cancer Center, M.D. Anderson Hospital and Tumor Institute, Houston, Texas (15)
- VOLKER MELL, Botanisches Institut der Universität Marburg, Marburg, West Germany (201)
- MASAMI MURAMATSU, Department of Biochemistry, Tokushima University School of Medicine, Tokushima, Japan (221)
- TOSHIO ONISHI, Department of Biochemistry, Tokushima University School of Medicine, Tokushima, Japan (221)
- RAM PARSHAD, Department of Pathology, Howard University College of Medicine, Washington, D.C. (421)
- N. R. RINGERTZ, Institute for Medical Cell Research and Genetics, Medical Nobel Institute, Karolinska Institute, Stockholm, Sweden (339)
- WILLIAM S. RUNYAN, Department of Food and Nutrition, Iowa State University, Ames, Iowa (381)
- MARLENE SABBATH, Department of Pathology, Lenox Hill Hospital, New York, New York (435)
- TAKASHI SAKAMOTO,¹ Division of Radiation Biology, Faculty of Pharmaceutical Sciences, Kanazawa University, Kanazawa, Japan (97)
- R. J. SANDERSON, Webb-Waring Lung Institute, University of Colorado Medical Center, Denver, Colorado (1)
- STEVEN D. SCHIMMEL, Department of Biochemistry, University of South Florida, College of Medicine, Tampa, Florida (289)
- RUPERT SCHMIDT-ULLRICH, Tufts-New England Medical Center, Department of Therapeutic Radiology, Division of Radiobiology, Boston, Massachusetts (235)
- JÜRGEN M. SCHMITT, Institut für Botanik der Universität Düsseldorf, Düsseldorf, West Germany (177)
- DANIEL SCHÜMPERLI, Department of Pharmacology and Biochemistry, School of Veterinary Medicine, University of Zürich, Zürich, Switzerland (89)
- HORST SENGER, Botanisches Institut der Universität Marburg, Marburg, West Germany (201)
- E. SIDEBOTTOM, Sir William Dunn School of Pathology, Oxford University, Oxford, England (339)

¹ *Present address:* Research Laboratories, NISSUI Pharmaceutical Co., Ltd., 1805 Inaricho, Souka-shi, Saitama 340, Japan.

JOHANN SONNENBICHLER, Max-Planck-Institut für Biochemie, Martinsried, West Germany (149)

WILLIAM G. TAYLOR, Cell Physiology and Oncogenesis Section, Laboratory of Biochemistry, National Cancer Institute, Bethesda, Maryland (421)

J. E. THOMPSON, Department of Biology, University of Waterloo, Waterloo, Ontario, Canada (303)

P. ROY VAGELOS, Merck, Sharp & Dohme Research Laboratories, Rahway, New Jersey (289)

DONALD F. H. WALLACH, Tufts-New England Medical Center, Department of Therapeutic Radiology, Division of Radiobiology, Boston, Massachusetts (235)

WAYNE WRAY, Department of Cell Biology, Baylor College of Medicine, Houston, Texas (111)

I. ZETL, Max-Planck-Institut für Biochemie, Martinsried, West Germany (149)

PREFACE

Volume XV of this series continues to present techniques and methods in cell research that have not been published or have been published in sources that are not readily available. Much of the information on experimental techniques in modern cell biology is scattered in a fragmentary fashion throughout the research literature. In addition, the general practice of condensing to the most abbreviated form materials and methods sections of journal articles has led to descriptions that are frequently inadequate guides to techniques. The aim of this volume is to bring together into one compilation complete and detailed treatment of a number of widely useful techniques which have not been published in full detail elsewhere in the literature.

In the absence of firsthand personal instruction, researchers are often reluctant to adopt new techniques. This hesitancy probably stems chiefly from the fact that descriptions in the literature do not contain sufficient detail concerning methodology; in addition, the information given may not be sufficient to estimate the difficulties or practicality of the technique or to judge whether the method can actually provide a suitable solution to the problem under consideration. The presentations in this volume are designed to overcome these drawbacks. They are comprehensive to the extent that they may serve not only as a practical introduction to experimental procedures but also to provide, to some extent, an evaluation of the limitations, potentialities, and current applications of the methods. Only those theoretical considerations needed for proper use of the method are included.

Finally, special emphasis has been placed on inclusion of much reference material in order to guide readers to early and current pertinent literature.

DAVID M. PRESCOTT

CONTENTS

LIST OF CONTRIBUTORS

xv

PREFACE

xviii

1. *Cell Separations by Counterflow Centrifugation*

R. J. Sanderson and Karyn E. Bird

I. Introduction	1
II. Principles of Counterflow Centrifugation	2
III. Theory of the Mechanics of Counterflow Centrifugation	3
IV. Derivation of a Suitable Chamber Shape	7
V. Application of Counterflow Centrifugation to Cell Separations	8
References	14

2. *Separation of Spermatogenic Cells and Nuclei from Rodent Testes*

Marvin L. Meistrich

I. Introduction	16
II. Principles of Separation	17
III. Preparation of Cell Suspensions	19
IV. Preparation of Nuclear Suspensions	22
V. Velocity Sedimentation	25
VI. Equilibrium Density Gradient Centrifugation	34
VII. Effect of Preparation of Suspension on Separation	36
VIII. Discussion	39
IX. Applications	48
X. Conclusions	52
References	52

3. *Separation of Mammalian Spermatids*

M. Loir and M. Lanneau

I. Introduction	55
II. Separation According to Cell Size	56
III. Separation According to Buoyant Density	67

IV. Discussion	76
References	77
4. <i>Isolation of Skeletal Muscle Nuclei</i>	
<i>LeRoy Kuehl</i>	
I. Introduction	79
II. Concentration of Nuclei in Skeletal Muscle	82
III. Homogenization	83
IV. Purification on Sacrose Gradients	83
V. Stability of Nuclei	84
VI. Detailed Procedures for Isolating Muscle Nuclei	84
References	88
5. <i>Isolation of Neuronal Nuclei from Rat Brain Cortex, Rat Cerebellum, and Pigeon Forebrain</i>	
<i>Clive C. Kuenzle, Alois Knüsel, and Daniel Schümperli</i>	
I. Introduction	89
II. Materials and Methods	90
III. Results and Discussion	90
IV. Conclusion	95
References	95
6. <i>Isolation of Metaphase Chromosomes from Synchronized Chinese Hamster Cells</i>	
<i>Masakatsu Horikawa and Takashi Sakamoto</i>	
I. Introduction	97
II. Cell Line, Medium, and General Techniques	98
III. Optimum Concentration of Colcemid and Duration of Treatment for Accumulating Mitotic Cells	99
IV. Collection of a Large Highly Purified Mitotic Cell Population by Repeated Treatments with a Combination of Colcemid and Harvesting Techniques	100
V. Biological and Cytological Effects of Single and Repeated Treatments with Colcemid and of Chilling on Chinese Hamster Cells	102
VI. Isolation of Metaphase Chromosomes from Mitotic Cell Population	105
VII. Discussion	106
References	109

7. *Use of Metrizamide for Separation of Chromosomes* Wayne Wray

I. Introduction	111
II. General Methods	112
III. Chromosome Characterization	116
IV. Chromosome Separation	122
V. Discussion	124
References	125

8. *Isolation of Interphase Chromatin Structures from Cultured Cells*

R. Hancock, A. J. Faber, and S. Fakan

I. Introduction	127
II. Isolation and Purification of Chromatin Structures	129
III. Events during Lysis	132
IV. Properties of Chromatin	136
V. Applications	143
References	146

9. *Quantitative Determination of Nonhistone and Histone Proteins in Chromatin*

Johann Sonnenbichler, F. Machiuno, and I. Zatl

I. Introduction	150
II. Existing Methods for Quantitative Determination of the Nonhistone Moiety in Nucleoprotein Material	150
III. Cello gel Electrophoresis for Quantitative Nonhistone Determinations	151
IV. Examples of the Quantitative Determination of Histones and Non-histones by Cello gel Electrophoresis	157
V. Discussion	160
References	161

10. *Solubilization of Chromatin with Heparin and the Isolation of Nuclear Membranes*

Michel Bornens

I. Introduction	163
II. Isolation of Nuclei	164

III.	Solubilization of Chromatin	164
IV.	Isolation of Nuclear Envelopes	170
V.	Summary	174
	References	174
11.	<i>Fractionation of Cell Organelles in Silica Sol Gradients</i>	
	<i>Jürgen M. Schmitt and Reinhold G. Herrmann</i>	
I.	Introduction	177
II.	Use of Silica Sols	179
III.	Fractionation of Organelles	182
IV.	Purity and Functionality of Silica-Purified Organelles	195
	References	199
12.	<i>Preparation of Photosynthetically Active Particles from Synchronized Cultures of Unicellular Algae</i>	
	<i>Horst Senger and Volker Mell</i>	
I.	Introduction	201
II.	Organisms, Growth Conditions, Synchronization	203
III.	Particle Preparation	203
IV.	Particle Characterization	209
V.	Photosynthetic Reactions of the Particles	209
VI.	Evaluation of Reproducibility of Particle Preparations and Activity	217
	References	218
13.	<i>Rapid Isolation of Nucleoli from Detergent-Purified Nuclei of Tumor and Tissue Culture Cells</i>	
	<i>Masami Muramatsu and Toshio Onishi</i>	
I.	Introduction	221
II.	Isolation of Nuclei from Various Cells with Detergents	223
III.	Isolation of Nucleoli from Detergent-Purified Nuclei	225
IV.	Applications	230
V.	Commentary	233
	References	234

14. *Isolation of Plasma Membrane Vesicles from Animal Cells*

Donald F. H. Wallach and Rupert Schmidt-Ullrich

I. General Introduction	235
II. Membrane Markers	238
III. Cell Disruption	247
IV. Centrifugal Fractionation of Membrane Vesicles	253
V. Aqueous Two-Phase or Liquid-Interface Partition	268
VI. Electrophoretic Techniques	270
VII. Concluding Remarks	273
References	273

15. *Rapid Isolation of Nuclear Envelopes from Rat Liver*

R. R. Kay and I. R. Johnston

I. Introduction	277
II. Isolation of Rat Liver Nuclei	279
III. Isolation of Nuclear Envelopes from Purified Nuclei	280
IV. Characterization of the Basic Nuclear Envelope Preparation	282
References	286

16. *Isolation of Plasma Membranes from Cultured Muscle Cells*

Steven D. Schimmel, Claudia Kent, and P. Roy Vagelos

I. Introduction	290
II. Cell Culture Conditions	290
III. Analytical Procedures	292
IV. Isolation of Plasma Membranes	293
V. Distribution, Purification, and Recovery of Membrane Markers	294
VI. Chemical Composition of Plasma Membranes	297
VII. Comments	298
References	300

17. *Preparation of Plasma Membranes from Amoebae*

J. E. Thompson

I. Introduction	303
II. Culture Methods	304
III. Plasma Membrane Isolation	305

IV.	Alternative Isolation Procedures	311
V.	Plasma Membrane Enzymes	314
VI.	Macromolecular Composition of Amoeba Plasma Membrane	315
VII.	Turnover of Plasma Membrane during Phagocytosis	318
	References	323
18.	<i>Induction of Mammalian Somatic Cell Hybridization by Polyethylene Glycol</i>	
	<i>Richard L. Davidson and Park S. Gerald</i>	
	I. Introduction	325
	II. Polyethylene Glycol Solutions	326
	III. Hybridization of Cells in Monolayers	327
	IV. Hybridization of Cells in Suspension	335
	References	337
19.	<i>Preparation of Microcells</i>	
	<i>T. Ege, N. R. Ringertz, H. Hamberg, and E. Sidebottom</i>	
	I. Introduction	339
	II. Micronucleate Cells	341
	III. Preparation of Microcells from Micronucleate Cells	347
	IV. Properties of Microcells	352
	V. Use of Microcells in Somatic Cell Genetics	356
	References	356
20.	<i>Nuclear Transplantation with Mammalian Cells</i>	
	<i>Joseph J. Lucas and Joseph R. Kates</i>	
	I. Introduction	359
	II. Preparation and Characterization of Karyoplasts	360
	III. Preparation and Characterization of Cytoplasts	365
	IV. Nuclear Transplantation	365
	References	369

21.	<i>Procedure for Preparation and Characterization of Liver Cells Made Permeable by Treatment with Toluene</i>	
	<i>Richard H. Hilderman and Peter J. Goldblatt</i>	
	I. Introduction	371
	II. Preparation of Liver Cells Made Permeable by Toluene	372
	III. Characterization of the Toluene-Treated Liver Cells	374
	IV. Conclusions	379
	References	380
22.	<i>Estimation of Intracellular Fluid Volume</i>	
	<i>William S. Runyan and Teresa H. Liao</i>	
	I. Introduction	381
	II. Procedure	382
	III. Results and Discussion	384
	References	385
23.	<i>Detection of Carbohydrates with Lectin-Peroxidase Conjugates</i>	
	<i>Nicholas K. Gonatas and Stratis Avrameas</i>	
	I. Introduction	387
	II. Methods of Isolation of Lectins and Preparation of Lectin-HRP Conjugates	388
	III. Fixation	392
	IV. Specificity of Binding between Lectin-HRP and Cell Surface or Intracellular Carbohydrate Moieties	392
	V. Topography, Mobility, and Endocytosis of Surface (Plasma Membrane), Lectin-HRP Conjugates	393
	VI. The Use of Lectin-HRP Conjugates for Demonstration of Intracellular Carbohydrate Moieties in Tissues	400
	VII. Comparison of Lectin-Ferritin to Lectin-HRP Conjugates	401
	VIII. Summary	403
	References	404
24.	<i>Measurement of the Growth of Cell Monolayers in Situ</i>	
	<i>Gerardo Martinez-López and L. M. Black</i>	
	I. Introduction	407
	II. Cells Used in Development of the Technique	408

III.	Handling of Cell Monolayers	408
IV.	Enumeration of Cell Nuclei	409
V.	Number of Fields in Which Counts Should Be Made	410
VI.	Use of the Technique for Testing the Suitability of Different Batches of a Component for a Medium	410
VII.	Estimation of Absolute Numbers of Cells	411
VIII.	Estimates of Specific Cell Populations from Nuclei on Reticule Points Alone	415
IX.	General Discussion	416
	References	419
25.	<i>Peptones as Serum Substitutes for Mammalian Cells in Culture</i>	
	<i>William G. Taylor and Ram Parshad</i>	
I.	Introduction	421
II.	Peptones as Serum Substitutes	423
III.	Cell Growth in Peptone-Containing Medium	428
IV.	Limitations and Prospects	430
	References	433
26.	<i>In Situ Fixation and Embedding for Electron Microscopy</i>	
	<i>Marlene Sabbath and Barbro Andersson</i>	
I.	Introduction	435
II.	Cells on Unsectionable Substrates (Flat-Faced Embedding Technique)	436
III.	Cells on Sectionable Substrates	438
	References	443
27.	<i>Genetic and Cell Cycle Analysis of a Smut Fungus (Ustilago violacea)</i>	
	<i>Joseph E. Cummins and Alan W. Day</i>	
I.	Introduction	445
II.	Biology and Culture	446
III.	Cell Biology	449
IV.	Genetic Methods	458
V.	Cell Cycle	464

CONTENTS

xiii

VI. Mutant Cell Cycle Controls References

467
469

SUBJECT INDEX

471

CONTENTS OF PREVIOUS VOLUMES

479

Chapter 1

Cell Separations by Counterflow Centrifugation

R. J. SANDERSON AND KARYN E. BIRD

*Webb-Waring Lung Institute,
University of Colorado Medical Center,
Denver, Colorado*

I. Introduction	1
II. Principles of Counterflow Centrifugation	2
III. Theory of the Mechanics of Counterflow Centrifugation	3
A. Notation and Coordinate System	3
B. A Derivation of the Equation of Motion of a Particle under Combined Centrifugal and Hydrodynamic Fields	4
IV. Derivation of a Suitable Chamber Shape	7
V. Application of Counterflow Centrifugation to Cell Separations	8
A. Age Distribution of Red Cells	9
B. Peripheral Blood Leukocyte Separation	10
C. Spleen Cell Separation	11
D. Fractionation of Cultured Cell Suspensions	13
References	14

I. Introduction

Counterflow centrifugation has been available as a technique for cell separation since 1948, when Lindahl first published an account of the method in *Nature* (Lindahl, 1948). In that same year, a patent was also filed in California by MacLeod (1948) for a counterflow centrifugation apparatus designed for a nonbiological application. More recently, Beckman Instruments developed and marketed a rotor which has been used by a number of investigators (Glick *et al.*, 1971; Flangas, 1974; Grabske *et al.*, 1974).

The original apparatus of Lindahl was exceedingly complex, and that of MacLeod was specialized for the concentration of particles of high specific gravity. The Beckman apparatus, however, is quite simple. We have modified